

CLAIMS:

1. A method for genotyping a subject with respect to a gene or target nucleic acid sequence associated with a pathological condition, said method comprising contacting an allele specific oligonucleotide immobilized to a solid support with a single-stranded form of RNA or DNA from a subject to be tested labeled directly or indirectly with a reporter molecule capable of giving an identifiable signal under conditions which permit hybridization of single stranded RNA or DNA which is exactly complementary to the immobilized allele specific oligonucleotide but substantially less or no hybridization of non-complementary single-stranded RNA or DNA molecules and then screening for the presence or absence or level of reporter molecule which provides an indicator of the genetic identity of the single-stranded RNA or DNA molecule which in turn provides the genotype of the subject.
2. The method of Claim 1 wherein the RNA or DNA from the test subject is directly labeled with labeled nucleotides incorporated *via* polymer chain reaction (PCR).
3. The method of Claim 1 wherein the RNA or DNA from the test subject is indirectly labeled with labeled nucleotides *via* hybridization of a labeled oligonucleotide to the test RNA or DNA.
4. The method of Claim 1 wherein the subject is selected for a human, a non-human primate, a livestock animal, a laboratory test animal, a companion animal and a captured wild animal.
5. The method of Claim 1 wherein the subject is a human.
6. The method of Claim 5 wherein the pathological condition is selected from an autoimmune disease, inflammatory condition, cancer, neurological disorder and a neurodegenerative disorder.

7. The method of claim 1 wherein the pathological condition is genetic deafness or a propensity for development of genetic deafness.

8. The method of Claim 1 wherein the pathological condition is associated with genetic deafness.

9. The method of Claim 1 wherein the hybridization step is under differential hybridization conditions which permits differential hybridization between identical nucleotide sequences and sequences having at least one mismatch and the identity of the genotype of the subject is determined by the presence, absence or level of signal from the reporter molecule.

10. The method of Claim 1 or 7 or 8 wherein the RNA or DNA is *connexin 26*, *pendrin*, mitochondrial 12S rRNA or *usherin*.

11. The method of Claim 1 wherein the hybridization conditions comprise hybridization in the presence of 1-4 X SSC at 30-50°C for 15-90 min followed by washing at 30-50°C in the following sequence:

1-4 X SSC/0.05% - 0.4% SDS (1-5 min);
0.1-1 X SSC/0.05% - 0.4% SDS (2-10 min);
0.5 X -5 X SSC (0.5-3 min);
2-8 X SSC/0.05% (0.5-3 min); and
2-8 X SSC/0.05%-2% Tween (0.5-3 min).

12. The method of Claim 1 wherein the immobilized oligonucleotides are from about 5 to about 100 nucleotides in length.

13. The method of Claim 12 wherein the immobilized oligonucleotides are from about 10 to about 30 nucleotides in length.

14. The method of Claim 12 wherein the immobilized oligonucleotides are from about

15 to about 30 nucleotides in length.

15. The method of Claim 12 wherein the immobilized oligonucleotides are selected from SEQ ID NO:1 to 64.

16. The method of claim 1 wherein a sequence of nucleotides is interrupted up- or down-stream of the immobilized oligonucleotide to improve hybridization sensitivity.

17. The method of Claim 16 wherein the interruption is in a sequence of G residues.

18. A method for genotyping a human subject from a gene or nucleic acid target selected from *connexin 26*, *pendrin*, mitochondrial 12S rRNA and *usherin* wherein a mutation in one or more of these genes or targets is indicative of genetic deafness or a propensity to develop genetic deafness, said method incorporating a label directly or indirectly into genomic DNA amplified from the human subject to be tested using primers which flank a DNA sequence corresponding to a potential mutation in a gene or nucleic acid target listed above and contacting single-stranded labeled forms of the amplified DNA with an immobilized oligonucleotide selected from SEQ ID NO:1 to SEQ ID NO:64 under stringency conditions such that substantially only identically complementary DNA from the subject is capable of hybridizing to the corresponding immobilized oligonucleotide and screening for hybridization by measuring a signal or level of signal from the label.

19. A method for genotyping a human subject from a gene or nucleic acid target selected from *connexin 26*, *pendrin*, mitochondrial 12S rRNA and *usherin* wherein a mutation in one or more of these genes or targets is indicative of genetic deafness or a propensity to develop genetic deafness, said method incorporating a label into genomic DNA amplified from the human subject to be tested using primers which flank a DNA sequence corresponding to a potential mutation in a gene or nucleic acid target listed above and contacting single-stranded labeled forms of the amplified DNA with an immobilized oligonucleotide selected from SEQ ID NO:1 to SEQ ID NO:32 under stringency conditions of 1-4 X SSC at 30-50°C for 15 min to 90 min followed by washing at 30-50°C

- 99 -

in the following sequence:-

1-4 X SSC/0.05% - 0.4% SDS (1-5 min);
0.1-5 X SSC/0.05% - 0.4% SDS (2-10 min);
0.5 X -5 X SSC (0.5-3 min);
2-8 X SSC/0.05% (0.5-3 min); and
2-8 X SSC/0.05%-2% Tween (0.5-3 min);

such that substantially only identically complementary DNA from the subject is capable of hybridizing to the corresponding immobilized oligonucleotide and screening for hybridization by measuring a signal or level of signal from the label.

20. A method for genotyping a human subject from a gene or nucleic acid target selected from *connexin 26*, *pendrin*, mitochondrial 12S rRNA and *usherin* wherein a mutation in one or more of these genes or targets is indicative of genetic deafness or a propensity to develop genetic deafness, said method incorporating a label into genomic DNA amplified from the human subject to be tested using primers which flank a DNA sequence corresponding to a potential mutation in a gene or nucleic acid target listed above and contacting single-stranded labeled forms of the amplified DNA with an immobilized oligonucleotide selected from SEQ ID NO:1 to SEQ ID NO:32 under stringency conditions of 1-4 X SSC at 30-50°C for 15 min to 90 min followed by washing at 30-50°C in the following sequence:-

1-4 X SSC/0.05% - 0.4% SDS (1-5 min);
0.1-6 X SSC/0.05% - 0.4% SDS (2-10 min);
0.5 X -5 X SSC (0.5-3 min);
2-8 X SSC/0.05% (0.5-3 min); and
2-8 X SSC/0.05%-2% Tween (0.5-3 min);

such that substantially only identically complementary DNA from the subject is capable of hybridizing to the corresponding immobilized oligonucleotide and screening for

- 100 -

hybridization by measuring a signal or level of signal from the label, wherein a GI value is determined by the algorithm:-

$$GI = \frac{SV_N}{SV_N + SV_M}$$

wherein:

SV_N is the normal spot value; and

SV_M is the mutant spot value;

such that:

if $0.8 < GI < 1.0$, then the genotype is N/N;

if $0.65 < GI < 0.5$, then the genotype is N/M; and

if $0.0 < GI < 0.2$, then the genotype is M/M;

wherein:

N is a normal allele; and

M is a mutant allele.

21. A set of one or more oligonucleotides having the sequence:-

$[n]_x - A$

wherein:

n is one or a range of different nucleotides;

x is the length of the nucleotide sequence [n]; and

A is a nucleotide sequence selected from SEQ ID NOs:33 to 64.

22. The set of one or more oligonucleotides of claim 21 wherein n is T.

- 101 -

23. The set of one or more oligonucleotides of claim 21 or 22 wherein x is from about 5 to about 30.
24. The set of one or more nucleotides of claim 21 wherein $[n]_x - A$ is selected from SEQ ID Nos: 1 to 32.
25. A kit comprising one or more oligonucleotides of any one of claims 21 to 24.